PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Jean-Luc GALZI

Serial No. 09/445,205

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Confirmation No. 9642

GROUP 1655

Examiner B. Sisson

USE OF A FLUORESCENT PROTEIN FOR DETECTING INTERACTION BETWEEN A TARGET PROTEIN AND ITS LIGAND

DECLARATION UNDER RULE 132

Commissioner for Patents

Washington, D.C. 20231

Sir:

I, Jean-Luc GALZI, hereby declare as follows:

My relevant background and experience are set forth in the attached C.V. I make this declaration in support of the present application, and to provide evidence in rebuttal of several contentions set forth in the Official Action of December 6, 2001.

I declare that one of ordinary skill in the art would be able to make and use the claimed invention based on the teachings provided in the present application. Furthermore, I declare that the present specification clearly indicates to one of ordinary skill in the art that at the time the application was filed, the inventors of the present application were in possession of the claimed invention.

The factual bases for my opinions in this regard are as follows:

In light of the specification, one of ordinary skill in the art would clearly appreciate that variants and fragments of a cnidarian autofluorescent protein include mutations which do not abolish the main property of the protein, namely that it is fluorescent.

The fluorescent property of the protein is defined as follows:

1) the protein should have a molecular extinction coefficient (ε) greater than about 14000 $M^{-1}.cm^{-1}$; and

2) its fluorescence quantum yield (Q) should be greater than about 0.4.

Changes in excitation and emission wavelength may be detected for variants or fragments. These modifications will be considered as acceptable if ε and Q are greater than 14000 and 0.4, respectively.

Variants of the fluorescent protein include all mutations in the DNA sequence that lead to a protein with a primary amino acid sequence identical to, or different from, the wild type sequence. In GFP for instance, out of the 240 amino acids that form the mature protein, only three are directly contributing to the formation of the fluorophore and about 10 stabilize the fluorophore. Many mutations done in the DNA sequence encoding GFP lead to fluorescent proteins with identical, different or no fluorescent properties. However, the invention is concerned with proteins which have the fluorescent

property as noted above. It is routine for one of ordinary skill in the art to determine which variants are appropriate for the invention.

Fragments of the fluorescent protein include deletions or additions of amino acids at both N-terminal and C-terminal extremities. These additions and deletions will be considered in the present context, if they do not alter fluorescence properties in such a way that ε and Q become smaller than defined above.

Variants and fragments can be obtained by random mutagenesis, by site-directed mutagenesis, or by using restriction endonucleases acting on the DNA. Random mutagenesis is obtained using experimental conditions of polymerase chain reaction such that the proof reading and corrections done by polymerizing enzyme are not done. Site-directed mutagenesis is carried out by polymerizing DNA from a primer oligonucleotide containing one or more mismatches with the template DNA.

Variants and fragments can be easily expressed in bacteria and purified using a one step purification procedure. The purity and quantity of the protein can then be determined. Using a defined amount of a purified variant or fragment, it is then possible to experimentally measure the ε value in absorbance per mole and per cm using the Beer-Lambert relationship. Fluorescence quantum yield can also be determined by exciting the protein at its maximal absorbance wavelength (determined using a spectrophotometer) and measuring emission at different

wavelengths. The emission spectrum can then be used to determine the fluorescence quantum yield of the variant or fragment by comparing its fluorescence relative to that of a reference such as fluoresceine.

Fusion proteins are easily obtained using a two step PCR (polymerase chain reaction).

This protocol consists in amplifying two DNA coding sequences (partners 1 and 2) with primers designed in such a way that each amplified partner can hybridize with the other one. The first PCR reaction consists in independent amplification of each partner coding sequences with extremities complementary to the other partner coding sequence. In a second step, the two PCR products having complementary cohesive ends are mixed together with primers allowing the amplification of the DNA stretch encompassing partner 1 and partner 2. The resulting large DNA fragment encodes a fusion protein comprising partner 1 and 2. The introduction of this DNA fragment into an expression vector allows expression of the fusion protein.

Fluorescence properties, in the context of the present patent application, refer to the autofluorescent protein and its variants or fragments. It means that when excited at a wavelength at which the protein absorbs light, the protein is able to emit light at longer wavelength. In the present application, the minimal absorption coefficient (ε) is approximately 18000 and the minimal value of the fluorescence quantum yield (Q) is

approximately 0.4.

The interaction is detected as a reduction in the amplitude of the donor's emission and/or an increase of the acceptor's emission, as a result of fluorescence resonance energy transfer. The amplitude of the reduction of donor's emission and/or increase of the amplitude of acceptor's emission is proportional to the concentration of ligand - target protein complex being formed, and reaches a plateau value when target protein is saturated with ligand.

The quantification of the interaction is carried out by determining the amplitude of the reduction of donor's emission and/or the increase of amplitude of acceptor's emission and by normalizing it to the maximal variation of amplitude of the donor's and/or acceptor's emissions. The degree of target protein binding sites is then calculated according to mass action law. Thus, one of ordinary skill in the art would clearly be able to make and use the presently claimed invention and clearly appreciate that applicants had possession of the claimed invention at the time the present application was filed.

The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Statute 1001 of Title XVIII of the United States Code and that such willful false statements

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may jeopardize the validity of the application or any patent issuing thereon.

June 4, 2002
(date)

Jean-Luc GALZI

CURRICULUM VITAE

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First name:	Jean-Luc
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Address:	Département Récepteurs et Protéines Membranaires, CNRS UPR 9050, Ecole Supérieure des Biotechnologies de Strasbourg, Bd Sébastien Brant, 67400 Illkirch
Tel:	(33) 3 90.24.47.59
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Position:	Research Director, National Center for Scientific Research (CNRS).

EDUCATION:

1987	Ph.D in Bio-organic chemistry, Louis Pasteur University, Strasbourg, France

POSITIONS:

1983-1987	fellow of the Ministère de la Recherche et de la Technologie.
1988	Post-doc Fellow of the Foundation for research at chemistry-biology interface (France)
1989-1990	Post-Doc Fellow of the French Association against Neuromuscular Diseases
1990	Tenure Position at the CNRS, Molecular Neurobiology, Pasteur Institute, Paris, France. Director: Prof. J.P. Changeux
1996	Group leader, Dpt. Receptors and Membrane Proteins, School of Biotechnology, Strasbourg. Director: Dr. F. Pattus

TEACHING

1987	DEA Molecular Chemistry and Physico-Chemistry, Nancy, Dir. Pr. G. Branlant.
1987-02	DEA Molecular Pharmacology, Strasbourg, Dir. Pr. C. Wermuth.
1990, 1991	DEA Structures et Evolution des Vertébrés, Paris VII, Dir. Pr.Clairambault
1992-1995	DEA Cellular and Molecular Pharmacology, Paris VI, Dir. Pr P. Ascher.
1993	DEA Biology and Health, Montpellier, Dir. Pr. J. Bockaert.
1995-02	Neurobiology Course, Biotechnology School, Strasbourg, Dir Pr. B. Kieffer.
1997-02	Neurobiology, Faculty of Pharmacy, Strasbourg, Dir Pr. A. Beretz.

RESEARCH ACTIVITIES

1983-1987:	Synthesis of photoactivatable probes and irreversible labeling of opioid receptors. supervisors: Profs M. Goeldner and C.G. Hirth.
1988-1995:	Post-doctoral research: Functional architecture of nicotinic acetylcholine receptors. Molecular Neurobiology, Pasteur Institute, Paris, France.
1996-	Dynamics of signal transduction mediatd by G protein-coupled receptors. CNRS UPR 9050, School of biotechnology, Strasbourg, France

RESEARCH SUPERVISION

1996	P. Alix, Post-graduate student. Diploma (June 1997) DEA Neurosciences (UPR CNRS9050).
1997-01	J.Y. Vollmer, Ph.D student, Strasbourg University.
1998-00	T. Palanché, Post-doc fellow
1998-0	M. Lima, Technician
1998-02	S. Zoffmann, PhD student
1999-0	S. Morisset, Post-doctoral fellow
1999-	B. Ilien, chargée de recherche INSERM
1998-	H. Matthes, chargé de recherches CNRS

1999- V. Utard, technician, CNRs

2000- C. Muller, PhD Student

2000- S. Lecat, Post doctoral fellow

INVITED ORAL PRESENTATIONS

1987/june CNRS-INSERM Research Center for Pharmacology et endocrinology, Montpellier, France.

1991/march Ecole normale supérieure, Paris

1991/Sept. Commissariat à l'énergie atomique (CEA), Saclay, France.

1991/june Fourth international symposium on neuromuscular diseases, Montpellier, France.

1992/may 25th Jerusalem symposium on quantum chemistry and biochemistry, Jerusalem.

1992/july. EMBO-INSERM Course: Current Methods in Membrane Protein Research. Le Vésinet, France.

1992/oct. Journées de l'Institut de Biologie, Collège de France, Paris.

1992/nov. Department Conference, Pasteur nstitute, Paris.

1992/nov Bio-Chromatography society, Paris.

1993/apr European Research Conference on Molecular Neurobiology Regulation and Biosynthesis and Function of Neuroreceptors and ionic channels. Aghia Pelaghia, Crete.

1993/May Science et Defense 1993: Biotechnologies in Life Sciences. La Villette, Paris.

1993/May Marion Merrel Dow. 20th anniversary Strasbourg Center. Palais des Congrès, Strasbourg.

1993/May Plenary Lecture, Neurochemistry Institute, Strasbourg, France.

1993/june Colloquium of the French Neuroscience Society: Receptors and transduction mechanisms. Montagnac (France).

1993/aug XXXIInd International Congress of Physiological Sciences Glasgow (UK).

1993/nov Ecole normale supérieure, Paris.

1994/mar USGEB-symposium: "Ion channels", Berne, Switzerland.

1994/Apr University of Paris XI, Orsay.

1994/july EMBL Heidelberg: Practical course on "Methods in membrane protein research".

1994/Sept 16th international Congress of Biochemistry and Molecular Biology (IUBMB) New Dehli, Inde.

1994/oct	Conférence Philippe Laudat/INSERM: Neuronal Nicotinic Acetylcholine Receptors: Diversity, Functions and Pathological implications, Bischenberg, France.
1995/jan	Department of pharmacology, Zürich University, Zürich, Switzerland.
1995/jan	Recent advances in Neurobiology VII, Japan Intractable Diseases Research Foundation, Tokyo, Japan.
1995/may	Department of Physiology, Genèva, Switzerland.
1995/may	French Neuroscience Society, Lyon, France.
1995/sept	Colloquuium on Biomembranes, GEIMM-GFB Frenc Biophysics Society, Toulouse, France.
1995/dec	XXIII^{eme} Congress of the French Physiological Society, Strasbourg, France
1996/mar	Institute of Pharmacology and Structural Biology, Toulouse, France.
1997/mar	International symposium on molecular biology of the synapse: from electric organ to brain, Institut Pasteur, Paris
1997/Sept	European Conference Scientiae Europeae, La Baule, France.
1997/sept	Synthélabo Biomoléculaire Research Center
1997/dec	Colloquium ULP/JAPAN Neurosciences, Cognisciences, Strasbourg, France.
1998/jan	Structural Biology Institute, Toulouse, France.
1998/mar	Fournier Research Center, Dijon.
1998/sept	University René Descartes (PARIS V), Faculty of Pharmacy.
1999/janv	Université Louis Pasteur Strasbourg.
1999/fev	Institut Theodor Kocher, Bern, Suisse.
1999/fev	G protein-coupled receptors Workshop, Princeton, USA
1999/mars	Association Française de Cytométrie, Institut Curie, Paris, France.
1999/mars	Gordon Research Conference on ligand recognition and molecular gating, Ventura, USA.
1999/mai	Séminaires Grenoblois de Neurosciences, Institut Albert Bonniot, Grenoble
1999/juil	Conference Collège de France (P. Corvol)
2000/mars	RECOB 8 (Rencontres de Chimie Organique Biologique) Aussois, France

2000/mai	Conference Chaire de Chimie, Collège de France (J.M. Lehn)
2000/oct	French Society for Endocrinology (Brest)
2000/oct	international Congress Tachykinin 2000, La Grande Motte (France)
2000/oct	Conference Institut de Pharmacologie et de Biologie Structurale (Toulouse)
2001/avr	Symposium GPCR, Collège de France, Paris.
2001/mai	University of Geneva, Department of Biochemistry
2001/juin	Institut Curie, Journée thématique fluorescence
2001/juin	Ecole Normale Supérieure (Paris).
2001/sept	Institut de Biologie Moléculaire des Plantes (Strasbourg)
2001/Sept	Molecular interactions on a micro- and nanometer scale Meiringen, Switzerland
2001/oct	Faculty of pharmacy, Copenhaguen, Denmark
2001/dec	Max Plank Institute for Brain Researcn, Francfort, Germany

RESEARCH RESPONSABILITIES

1991 reviewer of grant applications to CNRS (Section 20), France.

1993, 1996 External referee for Ph.D Thesis defense: Field: Biochemistry; Candidate: Marie-Hélène Fulachier, University Pairs XI (1993); candidate: Valéie Winkler-Dietrich, University Pairs XI (1996)

1992 Referee for grant applications to MRC (UK)

1994 Referee for Summer School Grant Applications to NATO.

1994 Member of the organizing comittee of a Conference Philippe LAUDAT: Neuronal Nicotinic Acetylcholine Receptors: Diversity, Functions and Pathological implications, Bischenberg, France.

1996- Member of the Scientific board of the Biotechnology School, Strasbourg.

1996-1999 Organizer of monthly Conferences at the Shool of Biotechnology.

1998- Member of the 25th Commission of the National Scientific Commitee of the CNRS

AWARDS

1994 CNRS brass medal

1997 Price Victor Noury, Thorlet, Henri Becquerel, Jules et Augusta Lazare of Cellular and Molecular Biology, French Academy of Sciences

PUBLICATIONS

1. Galzi, J.L., Ilien, B., Simon, E.J., Goeldner, M.P. & Hirth, C.G (1987) Marquage irréversible des récepteurs des opioides à l'aide de sels d'aryldiazonium dérivés du fentanil. **Tetrahedron Lett.**, 28, 401-404.

2.Galzi, J.L (1987) Synthèse de sondes photoactivables et marquage irreversible du récepteur des opioides. Thèse de doctorat de l'Université de Strasbourg I.

3. Ilien, B., Galzi, J.L., Méjean, A., Goeldner, M.P. & Hirth, C.G (1988) A mu-opioid receptor-filter assay: Rapid estimation of binding affinity of ligands and reversibility of long-lasting ligand-receptor complexes. **Biochem. Pharmacol.**, 37, 3843-3851.

4. Giraudat, J., Galzi, J.L., Revah, F., Changeux, J.P., Haumont, P.Y., Lederer, F. (1989). The noncompetitive blocker chlorpromazine labels segment MII but not segment MI on the nicotinic acetylcholine receptor alpha-subunit. **FEBS Lett.** 253, 190-198.

5. Galzi, J.L., Méjean, A., Ilien, B., Mollereau, C., Meunier, J.C., Goeldner, M.P. & Hirth, C.G. (1990). Photoactivatable opiate derivatives as irreversible probes of the mu-opioid receptor. **J. Med. Chem.**, 33, 2456-2464.

6. Galzi, J.L., Méjean, A., Goeldner, M.P., Hirth, C.G. & Ilien, B. (1990). Photoaffinity labelling of the mu-opioid receptor is dependent on the nature of the photosensitive group of carfentanil derivatives. **Eur. J. Pharmacol.**, 188, 321-328.

7. Revah, F., Galzi, J.L., Giraudat, J., Haumont, P.Y., Lederer, F., Changeux, J.P. (1990). The noncompetitive blocker [^{3}H]-chlorpromazine labels three amino acids of the acetylcholine receptor gamma subunit: Implications for the alpha-helical organization of the M2 segments and the structure of the ion channel. **Proc. Natl. Acad. Sci. USA.**, 87, 4675-4679.

8. Galzi, J.L., Revah, F., Black, D., Goeldner, M., Hirth, C., Changeux, J.P. (1990). Identification of a novel amino acid alpha-Tyr 93 within the active site of the acetylcholine receptor by photoaffinity labeling: additional evidence for a three-loop model of the acetylcholine binding site. **J. Biol. Chem.** 265, 10430-10437.

9 Changeux JP, Benoît P, Bessis A, Cartaud J, Devillers-Thiéry A, Fontaine B, Galzi JL, Klarsfeld A, Laufer R, Mulle C, et al (1990) Regulation of acetylcholine receptor gene expression by neural factors and electrical activity during motor endplate formation. **Biochem Soc Symp**, 56, 9-12

10 Changeux JP, Benoit P, Bessis A, Cartaud J, Devillers-Thiery A, Fontaine B, Galzi JL, Klarsfeld A, Laufer R, Mulle C, et al (1990) The acetylcholine receptor: functional architecture and regulation. **Adv Second Messenger Phosphoprotein Res**, 24, 15-9

11. Revah, F., Bertrand, D., Galzi, J.L., Devillers-Thiéry, A., Mulle, C., Hussy, N., Bertrand, S., Ballivet, M. & Changeux, J.P. (1991). Mutations in the channel domain alter desensitization of a neuronal nicotinic receptor. **Nature**, 353, 846-849.

12. Galzi, J.L., Revah, F., Bessis, A., Changeux, J.P. (1991). Functional architecture of the nicotinic acetylcholine receptor: From electric organ to brain. **Ann. Rev. Pharmacol. Toxicol.** 31, 37-72.

13. Galzi, J.L., Revah, F., Bouet, F., Ménez, A., Goeldner, M., Hirth, C., Changeux, J.P. (1991). Allosteric transitions of the acetylcholine receptor probed at the amino acid level with a photolabile cholinergic ligand. **Proc. Natl. Acad. Sci. USA.** 88, 5051-5055.

14. Galzi, J.L., Bertrand, D., Devillers-Thiéry, A., Revah, F., Bertrand, S., Changeux, J.P. (1991). Functional significance of aromatic amino acids from three peptide loops of the alpha7 neuronal nicotinic receptor site investigated by site-directed mutagenesis. **FEBS Lett.** 294, 198-202.

15. Galzi, J.L. & Changeux, J.P. (1991) The nicotinic acetylcholine receptor: A member of the superfamily of ligand gated ion channels. in **Biological Signal Transduction** NATO ASI Series, H52, pp 1-16.

16. Changeux, J.P., Devillers-Thiéry, A., Galzi, J.L. & Revah, F. (1992). The acetylcholine receptor: A model of allosteric protein mediating intercellular communication. in **Interactions among cell signalling systems**, CIBA Foundation Symposium N 164, Kobe, Japan, John Wiley and Sons eds. pp 66-89.

17. Bertrand, D., Devillers-Thiéry, A., Revah, F., Galzi, J.L., Mulle, C., Hussy, N., Bertrand, S. & Changeux, J.P. (1992). Unconventional pharmacology of a neuronal nicotinic receptor mutated in the channel domain. **Proc. Natl. Acad. Sci. USA.**, 89, 1261-1265.

18. Galzi, J.L., Devillers-Thiéry, A., Hussy, N., Bertand, S., Changeux, J.P. & Bertrand, D. (1992). Mutations in the channel domain of a neuronal nicotinic receptor convert its ion selectivity from cationic to anionic. **Nature**, 359, 500-505.

19. Changeux, J.P., Devillers-Thiéry, A., Galzi, J.L. & Bertrand, D. (1992). New mutants to explore nicotinic receptor function. **Trends in Pharmacol. Sci.**, 13, 299-301.

20. Galzi, J.L. & Changeux, J.P. (1992) The nicotinic acetylcholine receptor, a model of ligand-gated ion channel: Investigation of its functional organization by protein chemistry and site-directed mutagenesis. in **Membrane Proteins: Structures, Interactions and Models**, 25th Jerusalem syposium on quantum chemistry and biochemistry. A. Pullman et al. eds., Kluwer Academic Publishers. 127-146.

21. Galzi, J.L. & Changeux J.P. (1993) Les récepteurs-canaux de la membrane plasmique. in **Pharmacologie Moléculaire; Mécanisme d'action des médiateurs et des médicaments**, 2nd edition, (Landry & Gies eds) Arnette éditions, Chap 11, pp 269-303.

22. Devillers-Thiéry, A., Galzi, J.L., Bertrand, S., Changeux, J.P. & Bertrand, D. (1992). Stratified organization of the nicotinic receptor channel. **NeuroReport**, 3, 1001-1004.

23. Changeux, J.P., Galzi, J.L., Devillers-Thiéry, A. & Bertrand, D. (1992) The functional architecture of the acetylcholine nicotinic receptor

explored by affinity labeling and site-directed mutagenesis. **Quarterly Rev. Biophys.** 25, 395-432.

24. Aslanian, D., Grof, P., Galzi, J.L., Changeux, J.P. (1993) A Raman spectroscopic study of the acetylcholine receptor-rich membranes from T. marmorata- interactions of the receptor with carbamylcholine and d-tubocurarine. **Biochem. Biophys. Acta** 1148 (2), 291-302.

25. Galzi, J.L., Changeux J.P. (1993) Le récepteur nicotinique de l'acétylcholine: Une protéine allostérique impliquée dans la communication intercellulaire. dans **Entretiens "Science et Défense"** 1993. Biotechnologies dans les sciences de la vie. Dunod eds. (in press).

26. Bertrand, D., Galzi, J.L., Devillers-Thiéry, A., Bertrand, S., Changeux, J.P. (1993) Mutations that affect monovalent versus divalent cation permeability of a neuronal nicotinic receptor channel. **Proc. Natl. Acad. Sci. USA.** 90, 6971-6975.

27. Bertrand, D., Galzi, J.L., Devillers-Thiéry, A., Bertrand, S. & Changeux, J.P. (1993) Stratification of the channel domain in neurotransmitter receptors. **Current Opinion in Cell Biology,** 5, 688-693.

28. Devillers-Thiéry A., Galzi, J.L. Changeux, J.P., Bertrand, S. & Bertrand, D. (1993) Functional architecture of the nicotinic acetylcholine receptor: A prototype of ligand-gated ion channel **J. Memb. Biol.** 136, 97-112.

29. Eiselé J.L., Bertrand, S., Galzi, J.L., Devillers-Thiéry, A., Changeux, J.P. & Bertrand, D. (1993) Chimaeric nicotinic-serotoninergic receptor combines dictinct ligand binding and channel specificities **Nature**, 366, 479-483.

30. Galzi, J.L., Changeux, J.P. (1994) Ligand-gated ion channels as unconventional allosteric proteins. **Current opinion in Structural Biology**, 4, 554-565.

31. Galzi, J.L., Changeux, J.P. (1995) Neuronal nicotinic acetylcholine receptors: Molecular organization and regulations **Neuropharmacology** 34, 563-582

32. Corringer, P.J., Galzi, J.L., Eiselé, J.L., Bertrand, S., Changeux, J.P., Bertrand, D. (1995) Identification of a new component of the binding site of the nicotinic $\alpha 7$ homooligomeric receptor. **J. Biol. Chem.** 270, 11749-11752.

33. Galzi, J.L., Changeux, J.P. (1995) Biologie Moléculaire du récepteur nicotinique de l'acétylcholine **Archives Physiol. Biochem.** 103, D5-6.

34. Galzi, J.L. & Changeux, J.P. (1995) Ligand-gated ion channels as unconventional allosteric proteins. in **Challenges and perspectives in Neuroscience.** eds D. Ottoson, T. Bartfai, T. Hökfelt and K. Fuxe Wenner-Gren International series, Pergamon, 27-51.

35. Galzi, J.L., Edelstein, S.J. & Changeux, J.P. (1996) The multiple phenotypes of allosteric receptor mutants. **Proc. Natl. Acad. Sci. USA.** 93, 1853-1858.

36. Galzi, J.L., Bertrand, S., Corringer, P.J., Bertrand, D., Changeux, J.P. (1996) Identification of calcium binding sites which regulate potentiation of a neuronal nicotinic acetylcholine receptor. **EMBO J.** 15, 5824-5832.

37. Krause, R.M., Buisson, B., Bertrand, S., Galzi, J.L., Changeux, J.P. & Bertrand, D. (1998) Ivermectine: A positive allosteric effector of the neuronal alpha7 nicotinic acetylcholine receptors. **Mol. Pharmacol.** 53, 283-294.

38. Weill, C., Ilien, B., Goeldner, M., Galzi, J.L. (1999) Fluorescent muscarinic EGFP-hM1 chimeric receptors: design, ligand-binding and functional properties **J. Rec.Sign. Transd. Res.**, 19, 423-436.

39. Weill, C., Galzi, J.L., Chasserot-Golaz, S., Goeldner, M. and Ilien, B. (1999) Functional characterization and potential applications for EGFP- and epitope-fused human M1 muscarinic receptors **J. Neurochem.** 73, 791-801.

40. Corringer, P.J., Bertrand, S., Galzi, J.L., Devillers-Thiéry, A., Changeux, J.P., Bertrand, D. (1999) Mutational analysis of the selectivity filter of the alpha7 nicotinic acetylcholine receptor **Neuron**, 22, 831-43.

41.Corringer, P.J., Bertrand, S., Galzi, J.L., Devillers-Thiéry, A., Changeux, J.P., Bertrand, D. (1999) Molecular basis of the charge selectivity of nicotinic acetylcholine receptor and related ligand-gated ion channels. **Novartis Found Symp.** 225, 215-224

42. Vollmer, J.Y., Alix, P., Chollet, A., Takeda, K., Galzi, J.L. (1999) Subcellular compartmentalization of activation and desensitization of responses mediated by NK2 neurokinin receptors. **J.Biol. Chem**, 274, 37915-37922.

43. Haiech, J., Galzi, J.L. & Hibert, M.(2000) In ' ' **CALCIUM The Molecular Basis of Calcium Action in Biology and Medicine** ' ', R Pochet Ed., 2000, Kluwer Academic Publisher 641-646.

44. Zoffmann, S., Turcatti, G., Galzi, J.L. Dahl, M. & Chollet, A. (2000) Synhesis and characterization of fluorescent and photoactivatable MIP-1 alpha ligands and interactions with chemokine receptors CCR1 and CCR5. **J. Med. Chem.** 44, 215-222.

45. Palanche, T., Ilien, B., Zoffmann, S., Reck, M.P., Bucher, B., Edelstein, S., Galzi, J.L.(2001) Dual responses and desensitization of the NK2 tachykinin receptor are mediated by multiple conformational states as revealed by rapid kinetic measurements. **J.Biol. Chem** 276, 34853-34861

46. Valenzuela-Fernandez, A., Palanche, T., Amara, A., Magerus, A., Altmeyer, R., Delaunay, T., Virelizier, J.L., Baleux, F., Galzi, J.L., Arenzana, F. (2001) Optimal inhibition of X4 HIV isolates by SDF-1α requires interaction with cell surface heparan sulfate proteoglycans. **J.Biol.Chem** 276, 26550-26558

47 Lukas, R., Lucero, L., Buisson, B., Galzi, J.L., Puchacz, E., Fryer, J.D., Changeux, J.P., Bertrand, D. (2001) Neurotoxicity of channel mutations in heterologously expressed alpha7-nicotinic acetylcholine receptors. **Eur. J. Neurosci.**, 13, 1849-1860

48. Rapid internalization and recycling of the human neuropeptide Y Y_1 receptor (2002) Hervé Gicquiaux, Sandra Lecat, Mireille Gaire, Alain Dieterlen, Yves Mély, Kenneth Takeda, Bernard Bucher and Jean-Luc Galzi **J.Biol.Chem** 277, 6645-6655.

PATENT

1997 French Patent n° 97.06977 (5 june 1997) for: "Utilisation of a fluorescent protein for the detection of target protein-ligand interactions. Inventors: Galzi , J.L. & ALix P.

1998 International extension of the French Patent n° 97.06977 with referecne WOB97 CNR FLU